REMARKS

Applicants appreciate the courtesies extended by Examiner Larry Helms during an interview on April 11, 2005 with Applicants' attorney Rodney J. Fuller. The comments appearing herein are substantially in accord with those presented and discussed during the interview.

Please note that all paragraph numbers used herein refer to the paragraph numbers as set forth in the published application (Pub. No. US 2002/0197270).

Claims 8-11 are amended, claims 1-7, 13-16 are cancelled, and new claims 18-28 are added herein. Thus, claims 8-12, and 17-28 are pending in this application. The amendments to the claims are fully supported by the original specification and claims. Specifically, the amendments to claim 8 -- directed to the length of the peptide -- are fully supported by the specification at paragraphs [0052-0059]. For example, at paragraph [0058] of the specification it states that the peptides of the invention preferably have "9-30 amino acid residues" and at paragraphs [0054-55] Applicants provide examples of peptides that are as short as 7 amino acid residues in length (see SEQ ID NOS:16 and 19).

The new claims are also fully supported. For example, new claim 18-19 are fully supported by original claim 9; new claims 20-27 -- directed to a pharmaceutical composition comprising the peptide of claim 8 -- at paragraphs [0062-65]; and new claim 28 is supported by the examples -- see paragraphs [0068-100]. No new matter has been added by the amendments made herein. Entry of the amendments at this time is therefore respectfully requested.

Applicants appreciate the Examiner's withdrawal of the rejection of claims 8-11 under 35 U.S.C. §112, second paragraph for indefiniteness based on the term "chemical derivatives." As pointed out in Applicants response, the term "chemical derivative is defined in the specification at paragraph [0044] in the filed application, or alternatively numbered paragraphs [0056-57] of the specification as published in Pub. No. US 2002/0197270.

Claims 8-11 and 17 were objected to for encompassing non-elected inventions.

Section 809.03 of the MPEP explains that "there are a number of situations that arise in which an application has claims to two or more properly divisible inventions, so that a requirement to restrict the application to one would be proper, **but presented in the same case are one or more claims (generally called "linking" claims) inseparable therefrom and thus linking together the inventions otherwise divisible.**"

"The most common types of linking claims, which, if allowed, act to **prevent** restriction between inventions that can otherwise be shown to be divisible, are

(A) genus claims linking species claims; . . . . " (emphasis added)

In the present application, all of the claims are dependent on independent claim 8 (the genus or "linking" claim), where all other species claims are dependent and linked to claim 8.

In this type of situation, wherein a linking claim is present, the restriction requirement is dependent on the non-allowance of the linking claim. If the linking claim is later found to be patentable the restriction requirement must be withdrawn and the nonelected species dependent on the linking claim should be rejoined and examined (see, MPEP sections 809.03-04). The election of a species is to assist the Examiner in conducting a prior art review of the claims, but such a restriction is not proper if a linking claim is found to be allowable (see, MPEP section 803).

This is the precise situation in the present application. Applicants invention is directed to a synthetic peptide capable of eliciting antibodies to p53. The peptides is 7-30 amino acid residues in length and contains a sequence of a CDR of the heavy or light chain of an anti p53 mAb. The dependent claims are directed to specific species of the invention. In view of this, Applicants respectfully request that the objection of claims 8-11 and 17 be withdrawn.

Claims 8-11, 12, and 17 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, as discussed on pages 3-4 of the final Office Action. Applicants respectfully traverse this rejection.

Applicants point out that the claims are directed to a synthetic peptide and not to a method of inducing an antigenic response. The claimed peptide is "7 to 30 amino acid residues in length and contains a sequence of a CDR of the heavy or light chain of an anti-p53 mAb, and salts and chemical derivatives thereof." The claimed peptide is capable of eliciting antibodies to p53 as taught by the specification. The specification teaches, and one skilled in the art would understand, that these peptides induce anti-p53 antibodies. Applicants demonstrated this using anti-p53 antibody titers (ELISA assays for anti-p53 antibodies) both in BALB/c and in C57BL/6 mice (see paragraphs [0045] and Example 4, paragraphs [0103-106] -- "[t]he incidence of mice developing IgG anti-p53 antibodies

(ELISA assay) was 8/10 and 7/10 for peptides V and VI, the CDR3-based peptides of mAb 240 and mAb 421, respectively"). The anti-p53 antibodies produced *in vivo* are presumably Ab3 anti-p53 antibodies as the peptides used to elicit the anti-p53 antibodies *in vivo* were based on the CDR domains of Ab1 anti-p53 antibodies (paragraph [0020] of the published application).

The phrase "anti-p53 antibodies" is clear on its face. One skilled in the art would understand what the term means and that the presence of "anti-p53 antibodies" can be easily tested for using standard ELISA techniques to p53 as shown by Applicants.

Therefore, Applicants respectfully request that this rejection be withdrawn.

Claims 8-11, 12, and 17 were rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement, as discussed on pages 4 of the final Office Action. Applicants traverse.

Applicants have amended claim 8 to further recite that the synthetic peptide is 7 to 30 amino acid residues in length. This is in addition to the previous requirement that the peptide contain a sequence of a CDR of the heavy or light chain of an anti-p53 mAb. Applicants teach and provide numerous examples in the specification of synthetic peptides that are 7 to 30 amino acid residues in length and contain a CDR of the heavy or light chain of an anti-p53 mAb that are capable eliciting antibodies to p53 -- see, for example, SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14 and SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:22; and SEQ ID NO:23. Applicants have provided sufficient teachings in the specification in conjunction with numerous examples, such that one skilled in the art would reasonably conclude that Applicants had possession of the presently claimed invention at the time of filing. Thus, Applicants request that this rejection be withdrawn as well.

Claims 8-11, 12, and 17 were rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement, as discussed on pages 5-6 of the final Office Action.

The Examiner states that "[i]n the instant case a peptide from the CDR of an anti-p53 antibody was used as an immunogen and according to the network theory produces anti-idiotype antibodies which mimic the antigen." The Examiner further states, that "[i]t is unclear if then the anti-id produces anti-p53 antibodies as stated in the example or if the anti-p53 antibodies are Ab1 antibodies."

For clarification, Applicants point out, that the specification teaches anti-p53 antibody production *in vivo*. The anti-p53 antibodies are presumably Ab3 antibodies having similar specificity to the original antigen as the Ab1 antibody from which the CDR sequences used in the claimed peptides were derived. The specification teaches and verifies the production of anti-53 antibodies *in vivo* using the claimed peptides by standard ELISA assay techniques for anti-p53 antibodies. For example, paragraph [0104] in Example 4 discloses production of anti-p53 Abs by SEQ ID NO:21 (peptide V) ("The incidence of mice developing IgG anti-p53 antibodies (ELISA assay) was 8/10 and 7/10 for peptides V and VI, the CDR3-based peptides of mAb 240 and mAb 421, respectively"). Table 4 also demonstrates that mice immunized with peptides V-IX rejected the Meth A tumor, thereby showing that fragments of CDR-based peptides can be used to induce anti-p53 immunity.

The Examiner further states that "[t]here is no showing that peptides that are chemically derivatized lead to the rejection of tumors." As pointed out above, the specification defines "chemical derivatives" as a peptide "contain[ing] additional chemical moieties not normally a part of the peptide," including "[c]ovalent modifications of the peptides." The specification explains that "[s]uch modification may be introduced into the molecule by reacting target amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues," and lists "esters, N-acyl derivatives, and the like" as examples. In addition, since the critical peptide sequences are present in salts and chemical derivatives of the present peptides, which provide the peptides in different chemical form only (i.e., in salt form or with additional chemical moieties), a person skilled in the art would readily understand that the salts and chemical derivatives of the peptides have the same immunological properties as the peptides themselves. Therefore, the specification is enabling as to a peptide that contains a CDR sequence from an anti-p53 antibody and is capable of eliciting an immune response to p53, as well as to salts and chemical derivatives thereof.

In addition, Applicants enclose other evidence of enablement. First, Applicants enclose a copy of a PCT Application -- International Publication No. WO 2004/074323 ("the '323 publication"), attached as Exhibit A. The '323 publication is titled, "Idiotypic Vaccine" and was filed on February 23, 2004, claiming priority to a February 21, 2003 filing. The '323 publication relates to idiotypic vaccine compositions for us in inducing immunity to p53. This vaccine is based on the exact same concept as taught by Applicants in the present application, namely an idiotypic vaccine comprising CDRs of human Abs

directed against p53. At page 5, lines 24-27 of the '323 publication, it states that "peptides derived from the CDRs of human anti-p53 antibodies results in the generation of Ab2. As part of the idiotype cascade Ab2 will lead to the generation of Ab3, *i.e.* antibodies directed against p53." This provides further evidence that one of skill in the art would recognize and understand that anti-p53 Abs generated upon vaccination with peptides containing CDR sequences taken from Ab1 antibodies to p53 are Ab3 antibodies. Applicants were the first to teach and demonstrate the effectiveness of such peptides.

As stated above, Applicants have taught and provided numerous examples. The '323 publication provides additional working examples further supporting enablement. The results of the *in vivo* experiments conducted in the '323 publication indicated both a humoral response as well as cell-mediated responses to the claimed peptides. "All six of the trial patients who underwent *in vivo* DTH testing were found to have response to the individual vaccine peptides (Table 3)" -- page 21, lines 7-8. In view of the results, applicants of the '323 publication concluded on page 22, lines 33-35, that they "[had] demonstrated that the CDR regions of human anti-p53 antibodies are capable of initiating humoral and cellular immunity in animals and in individuals with advanced malignancy."

As even further evidence of enablement, Applicants submit herewith a copy of a web page description of the Pentrys™ vaccine -- attached as Exhibit B. The Pentrys™ vaccine is a mixture of nine peptides derived from human the CDRs of anti-p53 antibodies. The peptides in the Pentrys™ vaccine are believed to be the same as those in the '323 publication at page 14. See also, Abstract, "Phase I clinical trial of a human idiotypic p53 vaccine in patients with advanced malignancy," Annals of Oncology 15:324-329, 2004 -- attached as Exhibit C.

According to the Pentrys™ vaccine web page, a Phase 1b/2a clinical trial on the Pentrys™ vaccine in humans was successfully completed in at St. Vincent's Hospital, Sydney. The Phase 1a trial on four patients ended with no evidence of drug related toxicity and initial evidence that the drug is immunogenic. The 1b/2a studies confirmed the safety of the vaccine and demonstrated a strong immune response in all 14 patients involved in the trial. These results and teachings provide still further evidence of enablement of the presently claimed invention.

In view of the Applicants' teaching and examples -- which are confirmed by the findings and results presented in the '323 publication and studies on the Pentrys™ vaccine -- one skilled in the art would be enabled to practice the presently claimed invention.

While some experimentation may be necessary, undue experimentation would not be required. Applicants therefore respectfully request that § 112, first paragraph, rejection be withdrawn.

Claims 8-10 and 12 were rejected under 35 U.S.C. §102(b) as being anticipated by Jannot et al. (BBRC 230:242-246, January 1997) as discussed on pages 6-7 of the final Office Action.

Jannot merely teaches that the single chain antibody scFv-421 specifically binds the tumor suppressor protein p53. Jannot does not teach the presently claimed invention.

The presently claimed invention is directed to a synthetic peptide that is 7 to 30 amino acid residues in length and contains a sequence of a CDR of the heavy or light chain of an anti-p53 mAb. The claimed peptides are capable of eliciting antibodies to p53. Nothing in Jannot teaches, or for that matter suggest, the presently claimed synthetic peptides. Applicants were the first to discover the claimed synthetic peptides and their ability to induce anti-p53 immunity.

In order for a reference to properly anticipate a claim, the reference must teach each and every element of the claim. In view of the fact that Jannot fails to teach each and every element of the presently claimed invention, Applicants respectfully request that this rejection be with drawn.

In view of the above amendments and arguments, Applicant now believes all claims to be in condition for allowance. If there are any questions, the Examiner is invited to call Allan Fanucci at (212) 294-3311 to expedite the allowance of all the claims in this application.

Respectfully submitted,

5/3/05
Date

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202-282-5838

Exhibit A

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 September 2004 (02.09.2004)

PCT

(10) International Publication Number
WO 2004/074323 A1

(51) International Patent Classification[7]: C07K 16/18, 16/44, A61K 39/395, A61P 37/02

(21) International Application Number: PCT/AU2004/000225

(22) International Filing Date: 23 February 2004 (23.02.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2003900767 21 February 2003 (21.02.2003) AU

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/074323 A1

(54) Title: IDIOTYPIC VACCINE

(57) Abstract: The present invention relates to idiotypic vaccine compositions for use in inducing immunity to p53. The invention preferably relates to a vaccine composition comprising a pharmaceutically acceptable carrier and at least one peptide, wherein the at least one peptide is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASKKG-Y_2 and X_3-MQGLQTPYT-Y_3 in which X_1, X_2, X_3, Y_1, Y_2 and Y_3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

IDIOTYPIC VACCINE

Field of the invention

The present invention relates to idiotypic vaccine compositions for use in inducing immunity to p53.

Background of the Invention

Mutation of the p53 tumor-suppressor gene occurs in almost 50% of human cancers, including colon (65%), lung (70%), stomach (45%), breast (30%) and head and neck (60%). p53 has three functional domains: an acidic N-terminal domain characteristic of transcription factors, a regulatory C-terminal domain and a central DNA-binding domain. Mutated p53 protein loses its normal function in regulating the cell cycle and apoptosis. Mutated p53 is not appropriately degraded and therefore accumulates in the cell cytoplasm and nucleus.

p53 is considered to be an excellent tumor associated antigen for active immunotherapy because: (1) Overexpression of p53 results in the high level display of p53 peptides on the surface of tumor cells in association with human Class I HLA molecules; (2) p53 is one of the most well-characterised antigens, its gene sequence is known, and the three-dimensional structure of a number of key regions of the protein have been determined allowing structural definition and mapping of the relevant unique antigenic sites on p53 that offer useful targets for humoral and cellular immunity. Another good reason to target the molecule is that 1) p53 peptides are not displayed on normal cells; 2) T cells specific for p53 exist in humans. Unfortunately a number of studies have demonstrated that p53 is not a very potent immunogen probably because the molecule is a self-protein. If tolerance to p53 can be broken there is a theoretical possibility that vaccination will trigger a strong autoimmune reaction useful in retarding tumour progression. An alternative to the use of p53 fragments, is to use antigenic mimics which are devoid of any undesired properties that cause immunological tolerance and yet retain the ability to induce p53 specific immune responses.

In this invention, we provide an idiotypic vaccine comprising CDRs of human antibodies directed against p53.

The idiotypic network hypothesis first proposed by Lindemann in 1973 and Jerne in 1974 described the immune system as a network of interacting antibodies and lymphocytes. In this hypothesis, an antibody Ab1 can be used to generate a series of anti-idiotype antibodies against Ab1, termed Ab2. Some of these Ab2 molecules,

termed Ab2β, can fit into the paratopes of Ab1 and act as a functional mimic of the three-dimensional structure of the tumor-associated antigen (TAA) identified by Ab1. The Ab2β can in turn induce specific anti-anti-idiotype antibodies (Ab3) and T cells (T3) that recognise the original tumor-associated antigen identified by Ab1.

The use of anti-idiotype antibody (Ab2β) as a vaccination strategy has several major advantages: (1) It is safer to use as it does not involve the use of tumor-derived material to induce anti-tumor immunity; (2) it could represent an effective method of breaking tolerance to self tumor antigens, as the epitope structure is now transformed into an idiotype determinant and is expressed in a different molecular environment; (3) anti-idiotype antibody may have a higher affinity than the original antigen to bind to class I HLA molecules. This will favour the expression on antigen-presenting cells (APC) that process the anti-idiotype and favours T-cell activation; (5) when human anti-idiotypic antibodies are used, they may have the advantage of stimulating more efficiently human immune effector cells and in particular T cells. Recently, several clinical trials have showed that anti-idiotype antibody is very well tolerated.

In International Patent Application No. WO 00/56770 the present inventors disclose a number of human antibodies directed against p53. In addition the amino acid sequence of these antibodies together with the DNA sequences encoding these antibodies is provided. The disclosure of this application is included herein by reference.

Summary of the invention

In a first aspect the present invention provides an idiotypic vaccine composition, the vaccine composition comprising a pharmaceutically acceptable carrier and at least one peptide, wherein the at least one peptide is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASMKG-Y_2 and X_3-MQGLQTPYT-Y_3, in which X1, X2, X3, Y1, Y2 and Y3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

In a second aspect the present invention provides an idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one peptide, the at least one peptide being characterised in that it competes with a peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT for binding to p53.

In a third aspect the present invention provides an idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one peptide, the at least one peptide being characterised in that an antibody raised against the peptide reacts with at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a fourth aspect, the present invention provides an idiotypic vaccine composition, the vaccine comprising a pharmaceutically acceptable carrier and at least one DNA molecule, the DNA molecule comprising a sequence encoding at least one peptide, wherein the at least one peptide is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASMKG-Y_2 and X_3-MQGLQTPYT-Y_3, in which X1, X2, X3, Y1, Y2 and Y3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

In a fifth aspect, the present invention provides an idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one DNA molecule, the DNA molecule comprising a sequence encoding at least one peptide, the at least one peptide being characterised in that it competes with a peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT for binding to p53.

In a sixth aspect the present invention provides an idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one DNA molecule, the DNA molecule comprising a sequence encoding at least one peptide, the at least one peptide being characterised in that an antibody raised against the peptide reacts with at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a seventh aspect the present invention consists in a method of inducing an anti-p53 idiotypic response in a subject, the method comprising administering to the subject the composition according to any one of the first, second, third, fourth, fifth or sixth aspects of the invention.

In an eight aspect the present invention provides a method of inducing immunity against a disease caused by expression of mutant p53, the method comprising administering to the subject the composition according to any one of the first, second, third, fourth, fifth or sixth aspects of the invention.

Brief description of the figures

Figure 1. Humoral responses to individual vaccine peptides as measured by ELISA over the course of the vaccination schedule. Individual graphs show the results for one trial subject (subject 012 - A, G and I; subject 014 – B; subject 015 - C, E and H and subject 016 - D, F and J). The serum reactivity with peptide 2 is shown in figures A-D and I, peptide 5 in figures E, F and J and peptide 8 is shown in G and H. Individual biotinylated vaccine peptides (5 μg/ml) were bound to streptavidin-coated plates (5 μg/ml) and serum binding antibodies were detected using either a goat anti-human IgG, IgA and IgM-specific secondary alkaline-phosphatase conjugate (0.12 μg/ml) (A-H) or a panel of murine anti-human IgA-, IgG1-, IgG2-, IgG3- and IgG4-, IgM specific antibodies (0.25 μg/ml) followed by a goat anti-mouse IgG alkaline phosphatase conjugate (0.12 μg/ml) (I and J). Serum dilutions were 1:50, except A, 1:100; G, 1:25; H, 1:25. Visit 1 – baseline; Visit 2 - 1 month after vaccination 1; Visit 3 - 1 month after vaccination 2; Visit 4 - 1 month after vaccination 3; Visit 5 - 1 month after vaccination 4; Visit 6 - 2 months after vaccination 4. Mean of triplicates ± one standard deviation is shown.

Figure 2. Serum antibody responses to the individual vaccine peptides (A) and to p53 (B) in one of two sheep immunized with the pooled vaccine as measured by ELISA. **A.** Individual biotinylated vaccine peptides (5 μg/ml) were bound to streptavidin-coated plates (5 μg/ml) and serum binding antibodies present 10 days after the boost were detected with an alkaline phosphatase conjugated donkey anti-sheep IgG-specific secondary antibody (0.12 g/ml). **B.** Purified recombinant p53 (10μg/ml) was bound to plates and serum binding antibodies present before (pre-immune serum) and after (post-immune serum) immunization, were detected as above. Mean of triplicates ± one standard deviation is shown.

Figure 3. Humoral response to p53 in trial subject 012 measured over the course of the vaccination schedule by ELISA. Purified recombinant p53 (10μg/ml) was bound to plates and serum binding antibodies were detected with either a goat anti-human IgG, IgA and IgM-specific secondary alkaline-phosphatase conjugate (0.12 μg/ml) (A) or a panel of murine anti-human isotype specific antibodies (0.25 μg/ml) followed by a goat anti-mouse IgG alkaline phosphatase conjugate (0.12 μg/ml) (B). Data shown uses serum diluted 1:100, while reactivity was still detected at dilutions of 1:400. The relative serum concentration was calculated by dividing the concentration of a pool of

two positive control sera giving the equivalent absorbance at 410 nm to the test sample, by the concentration of the test sample. Visit 1 – baseline; Visit 2 - 1 month after vaccination 1; Visit 3 - 1 month after vaccination 2; Visit 4 - 1 month after vaccination 3; Visit 5 - 1 month after vaccination 4; Visit 6 - 2 months after vaccination 4. Mean of triplicates ± one standard deviation is shown.

Figure 4. Cell-mediated response to peptide 5 in trial subject 016, measured over the course of the vaccination schedule. Patient PBMCs were stimulated with 10 μg/ml peptide 5, incubated *in vitro* for 6 days, pulsed with ^{3}H-thymidine for 18 hours and then proliferation was assessed by ^{3}H-thymidine incorporation. Visit 1 – baseline; Visit 2 - 1 month after vaccination 1; Visit 3 - 1 month after vaccination 2; Visit 4 - 1 month after vaccination 3; Visit 5 - 1 month after vaccination 4; Visit 6 - 2 months after vaccination 4.

Figure 5. Cell-mediated response to the vaccine in two subjects, 016 (A and B) and 017 (C and D) measured at baseline time points (A and C) and 1 month after vaccination 3 (B) or 4 (D). CFSE-FITC stained subject PBMCs were stimulated with 50μg/ml of pooled vaccine and incubated *in vitro* for 6 days. CD8, CD71 and CD3 markers were detected using fluorescein-conjugated antibodies. Percentages of CD3-, CD4- and CD71-positive cells that have specifically proliferated in response to *in vitro* stimulation with the vaccine are shown.

Detailed description

The present inventors have demonstrated that immunisation with particular peptides derived from the CDRs of human anti-p53 antibodies results in the generation of Ab2. As part of the idiotype cascade Ab2 will lead to the generation of Ab3, i.e. antibodies directed against p53. The CDRs identified and selected are unique in that they result in the generation of Ab2 (antibody that represents a p53 structural mimic) that in turn results in immune responses (humoral and cell mediated) to regions on the three dimensional structure of p53 valuable in practicing tumour cell killing and the invention. There will also be regions on p53, defined by recognition by other CDRs from human monoclonal antibodies (Ab1s) which although immunogenic, may produce immune responses (Ab2s) but would fail to achieve tumoricidal activity. Human monoclonal antibodies (Ab1s) can be created to almost any region of p53, as processing of the molecule during its clearance in the body exposes many epitopes that may not be immunologically relevant for targeting immunity in the form of a

vaccine. A key to the invention is the identification of p53 sequence regions with Ab1s (the human monoclonal antibodies) that provide a site to which a successful anti-idiotypic cascade can be targeted. Thus an important aspect of the invention is the selection of CDRs on Ab1s (as defined by particular p53 sequences to which they bind) that in turn elicit immunity, via the anti-idiotypic cascade, to regions of the mutant p53 molecule that are exposed for recognition by CTLs.

As will be readily appreciated, while the results described herein have been achieved using peptides of particular sequence, similar results may be achieved using peptides of differing sequence. The present invention also extends to analogues of the peptides of the present invention. The critical factor is that the peptide antigen elicits substantially the same Ab2 response as at least one of the specified peptides. Accordingly, the essential characteristic of the peptide antigen is that it elicits an antibody that reacts with at least one of the specified peptide antigens.

Accordingly, in a first aspect the present invention provides an idiotypic vaccine composition, the vaccine composition comprising a pharmaceutically acceptable carrier and at least one peptide, wherein the at least one peptide is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASMKG-Y_2 and X_3-MQGLQTPYT-Y_3, in which X1, X2, X3, Y1, Y2 and Y3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

In a preferred embodiment of the present invention

X_1 is absent or is AVYYC

X_2 is absent or is LEWVG

X_3 is absent or is GVYYC

Y_1 is absent or is WGQGT

Y_2 is absent or is RVTI

Y_3 is absent or is FGEGT.

In a further preferred embodiment the composition comprises at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a further preferred embodiment the composition comprises at least 2 of, and more preferably, all 3 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In another preferred embodiment the composition further comprises at least one peptide selected from the group consisting of LEWMGIINPSGGSANYAPKFKGRLTMS, KLLIHWASTRESGVPDR,

AGLFCQQYYTTPLTFGGGT, YFCSRVKAGGPDYWGQGT and LLIYLGSTRASGVPDR.

In a second aspect the present invention provides an idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one peptide, the at least one peptide being characterised in that it competes with a peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT for binding to p53.

By "compete" it is meant that in the presence of the peptide at the same concentration, the binding of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, or GVYYCMQGLQTPYTFGEGT to p53 is reduced by at least 50%. The peptide will bind to p53 with equivalent, better or up to two orders of magnitude weaker affinity.

Competition may be measured in any of a number of ways well known to persons skilled in the art. Preferably the level of competition is measured by labelling AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, or GVYYCMQGLQTPYTFGEGT and measuring the level of binding to p53 in the presence or absence of the test peptide.

In a third aspect the present invention provides an idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one peptide, the at least one peptide being characterised in that an antibody raised against the peptide reacts with at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

By "reacts" it is meant that the antibody raised against the peptide binds to at least one peptide selected from AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a preferred embodiment of the second and third aspects of the present invention, the peptide is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASMKG-Y_2 and X_3-MQGLQTPYT-Y_3, in which X1, X2, X3, Y1, Y2 and Y3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

In a further preferred embodiment of the second and third aspects of the present invention

X_1 is absent or is AVYYC

X_2 is absent or is LEWVG

X_3 is absent or is GVYYC

Y_1 is absent or is WGQGT

Y_2 is absent or is RVTI

Y_3 is absent or is FGEGT.

In a further preferred embodiment of the second and third aspects of the present invention the composition comprises at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a further preferred embodiment of the second and third aspects of the present invention the composition comprises at least 2 of, and more preferably, all 3 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In another preferred embodiment of the second and third aspects of the present invention the composition further comprises at least one peptide selected from the group consisting of LEWMGIINPSGGSANYAPKFKGRLTMS, KLLIHWASTRESGVPDR, AGLFCQQYYTTPLTFGGGT, YFCSRVKAGGPDYWGQGT and LLIYLGSTRASGVPDR.

In a further preferred embodiment of the first, second and third aspects of the present invention the composition further comprises an adjuvant.

The ideal immune response against cancer is generally considered to involve the induction of cytotoxic T cells and a Th1 helper response. Despite the availability of agents that facilitate the skewing of the immune response towards this outcome the selection of adjuvant for cancer vaccines involves a level of routine experimentation in selection of the appropriate adjuvant.

In addition to the adjuvant it is often the case that a small peptide (generally less than 30 amino acids) is linked to a larger molecule (the carrier) to improve its immunogenicity. The selection of suitable carrier and adjuvant is key to the preparation of a safe and acceptable vaccine for human use.

In the trial set out below granulocyte-macrophage colony stimulating factor (GM-CSF) was used as an adjuvant. This cytokine is thought to be useful by virtue of its ability to mobilize antigen presenting cells and up-regulation of MHC class I and II molecules. In the context of peptide vaccines GM-CSF tends to induce a mixed Th1/Th2 response. Other cytokine adjuvants such as IL-2 and IL-12 may also be used, however, these have also been associated with excessive adverse effects and may not be suitable for a vaccine that reaches routine clinical use.

Other commonly used adjuvants have a largely undefined mechanism of action and may exert their effects purely by physical means such as prolonging antigen presentation and enhancing antigen localisation. These include miscellaneous oil-emulsion technologies, ISCOMS, alum (aluminium hydroxide and aluminium phosphate), and liposomal delivery systems. Some adjuvants appear to act via pathogen-recognition receptors (PRRs) to induce various co-stimulatory events necessary for the immune response. These include CpG oligonucleotides, lipopolysaccharide (LPS), the muramyl dipeptides (such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP) and bacterial toxins such as cholera toxin. In the latter case the peptide could be conjugated to a bacterial toxin, a technique that is being trialed in human gastrointestinal malignancy with G17-DT, a conjugate of gastrin with diphtheria-toxin. All of these agents, to some degree influence polarisation of the immune response.

As with diphtheria toxin and cholera toxin the peptides may be conjugated with other bacterial toxins such as tetanus toxoid or proteins such as KLH (keyhole limpet haemocyanin).

Additional examples of adjuvants which may be effective include but are not limited to N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmit-oyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contain three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion.

Further examples of adjuvants and other agents include aluminium potassium sulfate (alum), beryllium sulfate, silica, kaolin, carbon, water-in-oil emulsions, oil-in-water emulsions, *Corynebacterium parvum* (*Propionobacterium acnes*), *Bordetella pertussis*, polyribonucleotides, sodium alginate, lanolin, lysolecithin, vitamin A, saponin, liposomes, levamisole, DEAE-dextran, blocked copolymers or other synthetic adjuvants. Such adjuvants are available commercially from various sources, for example, Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.) or Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Michigan). It is particularly preferred to include adjuvants which promote helper T cell responses, such as diphtheria, pertussis and tetanus toxins and ovalbumin. Other preferred adjuvants include immune stimulatory complexes (ISCOMs) which are small micelles of detergent such as Quil A. The immunogens of the invention may be present within

the micelles which can fuse with antigen-presenting cells, allowing the immunogen to enter the cytosol.

Polypeptides of the present invention, especially peptides, may also be prepared as self-adjuvanting peptides by conjugation to fatty acids, for example as described in WO93/02706.

It is presently preferred, however, that the adjuvant is GM-CSF.

In a fourth aspect, the present invention provides an idiotypic vaccine composition, the vaccine comprising a pharmaceutically acceptable carrier and at least one DNA molecule, the DNA molecule comprising a sequence encoding at least one peptide, wherein the at least one peptide is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASMKG-Y_2 and X_3-MQGLQTPYT-Y_3, in which X1, X2, X3, Y1, Y2 and Y3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

In a preferred embodiment of the fourth aspect of the present invention

X_1 is absent or is AVYYC

X_2 is absent or is LEWVG

X_3 is absent or is GVYYC

Y_1 is absent or is WGQGT

Y_2 is absent or is RVTI

Y_3 is absent or is FGEGT.

In a further preferred embodiment of the fourth aspect of the present invention the composition comprises a DNA molecule encoding at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a further preferred embodiment of the fourth aspect of the present invention the DNA molecule encodes at least 2 and more preferably all 3 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a further preferred embodiment of the fourth aspect of the present invention the DNA molecule comprises a further sequence encoding GM-CSF.

In another preferred embodiment of the fourth aspect of the present invention the DNA molecule comprises a further sequence encoding at least one peptide selected from the group consisting of LEWMGIINPSGGSANYAPKFKGRLTMS, KLLIHWASTRESGVPDR, AGLFCQQYYTTPLTFGGGT, YFCSRVKAGGPDYWGQGT and LLIYLGSTRASGVPDR.

In a fifth aspect the present invention provides an idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one DNA molecule, the DNA molecule comprising a sequence encoding at least one peptide, the at least one peptide being characterised in that it competes with a peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT for binding to p53.

In a sixth aspect the present invention provides an idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one DNA molecule, the DNA molecule comprising a sequence encoding at least one peptide, the at least one peptide being characterised in that an antibody raised against the peptide reacts with at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a preferred embodiment of the fifth and sixth aspects of the invention, the peptide encoded by the DNA molecule is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASMKG-Y_2 and X_3-MQGLQTPYT-Y_3, in which X1, X2, X3, Y1, Y2 and Y3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

In a preferred embodiment of the fifth and sixth aspects of the present invention

X_1 is absent or is AVYYC

X_2 is absent or is LEWVG

X_3 is absent or is GVYYC

Y_1 is absent or is WGQGT

Y_2 is absent or is RVTI

Y_3 is absent or is FGEGT.

In a further preferred embodiment of the fifth and sixth aspects of the present invention the composition comprises a DNA molecule encoding at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a further preferred embodiment of the fifth and sixth aspects of the present invention the DNA molecule encodes at least 2 and more preferably all 3 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

In a further preferred embodiment of the fifth and sixth aspects of the present invention the DNA molecule comprises a further sequence encoding GM-CSF.

In another preferred embodiment of the fifth and sixth aspects of the present invention the DNA molecule comprises a further sequence encoding at least one peptide selected from the group consisting of LEWMGIINPSGGSANYAPKFKGRLTMS, KLLIHWASTRESGVPDR, AGLFCQQYYTTPLTFGGGT, YFCSRVKAGGPDYWGQGT and LLIYLGSTRASGVPDR.

As will be recognised the composition of the fourth, fifth and sixth aspects of the present invention is for use in DNA vaccination.

The ability of direct injection of non-replicating plasmid DNA coding for viral proteins to elicit protective immune responses in laboratory and preclinical models has created increasing interest in DNA immunisation. A useful review of DNA vaccination is provided in Donnelly *et al*[1], the disclosure of which is incorporated herein by reference.

DNA vaccination involves the direct *in vivo* introduction of DNA encoding an antigen into tissues of a subject for expression of the antigen by the cells of the subject's tissue. DNA vaccines are described in US 5,939,400, US 6,110,898, WO 95/20660 and WO 93/19183, the disclosures of which are hereby incorporated by reference in their entireties. The ability of directly injected DNA that encodes an antigen to elicit a protective immune response has been demonstrated in numerous experimental systems (see, for example, Conry *et al*[2], Cardoso *et al*[3], Cox *et al*[4], Davis *et al*[5], Sedegah *et al*[6], Montgomery et *al*[7], Ulmer *et al*[8], Wang *et al*[9], Xiang *et al*[10], Yang *et al*[11], Ulmer *et al*[12], Wolff *et al*[13]).

To date, most DNA vaccines in mammalian systems have relied upon viral promoters derived from cytomegalovirus (CMV). These have had good efficiency in both muscle and skin inoculation in a number of mammalian species. A factor known to affect the immune response elicited by DNA immunization is the method of DNA delivery, for example, parenteral routes can yield low rates of gene transfer and produce considerable variability of gene expression[14]. High-velocity inoculation of plasmids, using a gene-gun, enhanced the immune responses of mice[15,16], presumably because of a greater efficiency of DNA transfection and more effective antigen presentation by dendritic cells. Vectors containing the nucleic acid-based vaccine of the invention may also be introduced into the desired host by other methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion,

DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), or a DNA vector transporter.

In other embodiments the DNA may be delivered by a virosome or liposome to skin or mucosa. Viral vectors, such as replication-defective adenoviridae, may also be used, and may be delivered by direct, non-invasive (needle-less) vaccination onto bare skin[17].

One of the advantages of a DNA vaccine is that the vector itself can act as an immunologic adjuvant. Firstly, intracellular expression of the antigen allows presentation by the major histocompatibility class I pathway and induction of a CD8+ve cytotoxic T lymphocyte response. Secondly, skewing towards a Th1-response may be enhanced by the use of bacterial DNA as it may contain a high content of unmethylated CpG motifs (e.g. GTCGTT)[18].

In a seventh aspect the present invention consists in a method of inducing an anti-p53 idiotypic response in a subject, the method comprising administering to the subject the composition according to any one of the first, second, third, fourth, fifth or sixth aspects of the invention.

In an eighth aspect the present invention provides a method of inducing immunity against a disease caused by expression of mutant p53, the method comprising administering to the subject the composition according to any one of the first, second, third, fourth, fifth or sixth aspects of the invention.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

All publications mentioned in the specification are herein incorporated by reference.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

In order that the nature of the present invention may be more clearly understood preferred forms thereof will be described with reference to the following non-limiting Examples.

Materials and Methods

Preparation of Pentrix

Peptide Manufacture

The production of non-GMP grade peptides (Table 1) was performed by Multiple Peptide Systems (MPS), San Diego, USA. MPS is a specialist unit that routinely produces peptides for numerous clinical trials.

	Antibody specificity	Derivation of CDR	Peptide Sequence
1	N - terminus	Heavy CDR2	H-LEWMGIINPSGGSANYAPKFKGRLTMS-OH
2	N - terminus	Heavy CDR3	H-AVYYCLLQALKHWGQGT-OH
3	N - terminus	Light CDR2	H-KLLIHWASTRESGVPDR-OH
4	N - terminus	Light CDR3	H-AGLFCQQYYTTPLTFGGGT-OH
5	Central domain	Heavy CDR2	H-LEWVGFIRSKAYGAATAYAASMKGRVTI-OH
6	Central domain	Heavy CDR3	H-YFCSRVKAGGPDYWGQGT-OH
7	Central domain	Light CDR2	H-LLIYLGSTRASGVPDR-OH
8	Central domain	Light CDR3	H-GVYYCMQGLQTPYTFGEGT –OH

Table 1: Peptide sequences of Pentrix™. These sequences correspond to the antigen binding regions of two human anti-p53 antibodies with amino (N) and central domain (C) specificity. The CDR sequences are underlined. Each peptide contains additional amino acids from the flanking framework regions from each side of the CDR. CDR = complementarity determining region.

The peptides were sterile with no detectable endotoxin (< 0.1 U/mg) and pure as assessed by RP-HPLC and amino acid sequence analysis. The vaccine was prepared by admixing the peptides (500 μg of each) with GM-CSF (100 μg; Schering-

Plough, Baulkham Hills, Australia) in 31.25% DMSO/31.25% saline/37.5% dH_20 (800 µl).

Subjects and clinical protocol

Ten subjects with metastatic malignancy (1 breast carcinoma, 5 colorectal carcinoma, 1 non-small cell lung carcinoma, 1 haemangiopericytoma, 1 renal cell carcinoma, 1 prostate carcinoma), 1 subject with local recurrence (squamous cell carcinoma of head and neck) and 3 subjects with definitively treated advanced malignancy who were disease free at study entry (1 esophageal, 1 renal and 1 non-small cell lung cancer) were enrolled onto the trial (median age = 52.5 yrs, range 39-70 yrs, 7 males and 7 females). Detailed informed consent was obtained from all of the patients in accordance with the St Vincent's Hospital Human Research Ethics Committee.

Eligible subjects were required to have Eastern Co-operative Oncology Group (ECOG) performance status 0 (11 subjects) or 1 (3 subjects), a life expectancy of at least 6 months, overexpression of p53 in either the primary or metastatic tumour (as indicated by moderate to strong staining of at least 20 % of tumour cells with DO7 anti-p53 antibody (Dako, Botany, NSW)) and a positive response to recall antigen as determined by the CMI multitest (Pasteur Merieux, Lyon, France). Subjects were excluded if they had undergone chemotherapy, radiotherapy or surgery or had received immunosuppressive therapy in the preceding 6 weeks.

The first group of subjects received one intradermal vaccination delivered as 4 separate injections of 200 µl. CMI testing of the second group of subjects was preceded by a tetanus booster. The second group then received four vaccinations at monthly intervals, delivered as for the first group. Blood for immunologic assays was drawn before each immunization and 1 and 2 months post-vaccination 4. Clinical observations, including temperature, blood pressure, heart rate and respiratory rate, were recorded at the time of injection and at 24 and 48 hours. Adverse events were graded according to the NCI-Common Toxicity Criteria Version 2[19]. Subjects who completed all four vaccinations were assessed for vaccine-specific immune responses. All patients were assessed for safety and toxicity. Tumor response was not assessed as part of this study.

Delayed type hypersensitivity (DTH) testing was carried out 1 month after the final vaccination. The individual vaccine peptides (100 µg), a negative control peptide derived from the light chain CDR2 sequence of a human HIV gp41-specific antibody (H-PKLLIYKASSLESGVPSR-OH) and vehicle only were injected intradermally to the

interscapular area. Induration of ≥ 10 mm^2 at 48 hours after injection was considered positive.

Immunization of sheep and rabbits with the vaccine

Polyclonal sera specific for each of the peptides were generated by immunizing two sheep and two rabbits. Sheep were primed with 200 μg of the peptide mixture in a CpG DNA adjuvant (ImmunEasy Adjuvant, Qiagen), boosted 6 weeks later and bled on the day of priming and 10 days after the boost. Polyclonal sera reactive with peptides 3 and 7 could not be generated in sheep and therefore rabbits were primed with a mixture of peptides 3 and 7 (375 μg each) in a QuilA/DEAE Dextran/Montanide ISA 50V adjuvant mixture (Bioquest, Sydney, Australia) and boosted 6 weeks later.

Humoral immune response to the vaccine and p53

ELISAs were used to detect serum antibodies specific for either the individual vaccine peptides or recombinant p53. Each biotinylated peptide (5 μg/ml; Auspep, Melbourne, Australia) was captured separately onto plates coated with 5 μg/ml of streptavidin (Sigma, St. Louis, MO) and the purified recombinant p53 (10 μg/ml) was directly plated as previously described[20]. Sera were applied in triplicate and tested over a range of dilutions (1:25 – 1:800). Binding antibodies were detected with an alkaline phosphatase-conjugated goat anti-human IgA+IgG+IgM (H+L) antibody (0.12 μg/ml; Jackson Immunoresearch, West Grove, PA). Samples with a mean change in $Abs_{410\,nm}$ (the $Abs_{410\,nm}$ with peptide - $Abs_{410\,nm}$ without peptide) greater than 0.3 were considered positive. Positive responses were isotyped by detecting binding antibodies with a panel of murine anti-human IgA-, IgG1-, IgG2-, IgG3- , IgG4-and IgM specific antibodies (0.25 μg/ml; Zymed, San Francisco, CA) followed by a goat anti-mouse IgG alkaline phosphatase conjugate (0.12 μg/ml; Jackson Immunoresearch, West Grove, PA). Detection of anti-peptide and anti-p53 responses in the rabbits and sheep were performed using ELISAs as described above, but serum binding antibodies were detected using alkaline phosphatase-conjugated donkey anti-sheep or anti-rabbit IgG antibodies (0.12 μg/ml; Jackson Immunoresearch, West Grove, PA). Sera from 54 cancer controls (median age = 66.3 yrs, age range = 40.9 - 84.0 yrs, 36 males and 18 females) and 30 normal controls (median age = 34.5 yrs, age range = 22.7 – 61.6 yrs, 14 males and 16 females) were also tested.

Cell-mediated response to the vaccine and p53

Proliferation in response to stimulation by vaccine (individual peptides or vaccine pool) or p53 was measured by (^{3}H)-thymidine and carboxyfluorescein succinimidyl ester (CFSE) proliferation assays, and the secretion of IFN-γ was measured by IFN-γ ELISPOT assay. Peripheral blood mononuclear cells (PBMCs) were plated at a concentration of 1 x 10^5 cells/well for thymidine proliferation and ELISpot assays and at 1 x 10^6 cells/well (24-well plates) for the CFSE proliferation assay. ELISpot plates were coated with mouse anti-human IFN-γ antibody (Diaclone, France) and cells for CFSE assay were stained with 5 μM CFSE-FITC (Molecular Probes, Eugene, OR). All PBMCs were in RPMI supplemented with 2 mM L-glutamine, 10mM HEPES, 100U/mL penicillin/streptomycin and 10 % human AB serum (Australian Red Cross Blood Service).

Cells were stimulated with the pooled vaccine (50 μg/ml), individual vaccine peptides (10 μg/ml), recombinant p53 (5 or 10 μg/ml), positive controls included phytohaemagglutinin (0.5 μg/ml; Murex, Dartford, UK) and Interleukin-2 (10 U/ml; Roche Diagnostics, Mannheim, Germany) and Staphylococcus enterotoxin B (1 μg/ml; Sigma-Aldrich, St Louis, Missouri) and negative control wells contained medium alone and cell alone. The recall antigens CMV lysate, (1/2000 dilution; BioWhittaker, Walkersville, MD) and tetanus toxoid (2.5 Lfu/ml; CSL) were also tested. PBMCs were incubated at 37 °C and 5 % CO_2 atmosphere for 20 hours (ELISpot) or 6 days (proliferation assays).

IFN-γ secretion was then detected according to the Diaclone protocol (Diaclone, France), and spots were counted using an automated ELISpot plate counter (Autoimmun Diagnostika, Strassberg, Germany). The frequency of antigen-specific T cells present was calculated by subtracting the mean number of spots obtained in the absence of antigen from the mean number of spots obtained in the presence of antigen. A sample was considered positive if the mean spot count was ≥ 50 per 10^6 PBMCs.

In the CFSE proliferation assay, cells were stained with fluorescein-conjugated monoclonal antibodies specific for CD3, CD71 (BD Biosciences, San Jose, CA) and CD8 (Beckman Coulter, Miami, Florida) and analysed using an EPICS-XL flow cytometer[21]. Staining was considered positive when the percentage of cells that were both CD71 positive and had moved out of the undivided population was at least twice that observed in the absence of stimulation.

In the thymidine proliferation assay, cells were pulsed with 0.5 μCi/well [methyl-^{3}H]-thymidine (Amersham, Buckinghamshire, UK) and harvested 18 hours

later. [^{3}H]-thymidine incorporation was measured by liquid scintillation counting. A sample was considered to be positive when the stimulation index[22] for each well in the quadruplicate sample exceeded the mean of unstimulated cells by at least three standard deviations.

Results

Clinical outcomes

A total of 102 individuals were screened for entry into this study, forty-two were excluded on clinical grounds and a further 26 individuals were excluded because their tumor did not over-express p53. Twenty-one subjects were subjected to CMI testing and seven of these were excluded due to a lack of demonstrable CMI response to common recall antigens including tetanus toxoid. Four subjects participated in the single-dose phase of the study (subjects 002, 004, 006 and 007) and ten in the multi-dose phase (009, 010, 011, 012, 014, 015, 016, 017, 018 and 019). Four of the multi-dose subjects (009, 010, 011, 019) were withdrawn before they completed all four vaccinations due to disease progression requiring alternative treatment.

Over the 49 separate occasions of vaccination, the vaccine was well-tolerated and the majority of recorded adverse events were attributable to the underlying disease. As expected, local reactions were the most common adverse event. Intradermal infiltration of 200 µl resulted in small blebs in all of the subjects, and a number of the early subjects developed blistering and ulceration after 24 to 48 hours. This local reaction was attributed to rapid precipitation of the GM-CSF and peptide mixture and was ameliorated by changing the diluent from saline to water and agitating the syringe between injections. Subjects who received multiple vaccinations developed an increasing area of post-vaccination induration after each occasion, suggestive of a local DTH response.

Toxicities that were probably or possibly attributed to the vaccine were limited to grades 1 or 2 in severity and included arthralgia (10 occasions), nausea (4 occasions) and febrile reactions (16 occasions). These effects have all been described with GM-CSF alone[23]. Grade 3 or 4 toxicities, including jaundice (1 occasion), motor neuropathy (2 occasions), vomiting (1 occasion), tumour pain (3 occasions), neuropathic pain (1 occasion) and non-neutropenic sepsis (1 occasion), and two serious adverse events, cord compression and death from pneumonia, were attributable to disease progression.

Humoral response to the vaccine

Sera from four of the six assessable trial subjects (012, 014, 015 and 016) were found to be reactive with peptide 2, two (012 and 015) were reactive with peptide 8 and two (015 and 016) were reactive with peptide 5 (Figure 1A-H). However, one of these responses was observed at the baseline timepoint (patient 015, visit 1) and was not augmented by vaccination (Figure 1E). No patient demonstrated a response to peptides 1, 3, 4, 6 or 7, although positive responses to each of these peptides were observed using the polyclonal sheep sera (Figure 2A) or rabbit sera (data not shown). The vaccine-specific humoral responses seen in humans were only detected after at least two vaccinations had been administered. Titres of peptide-specific serum antibodies rose to maximal levels one month after vaccination 4, then fell again in the next month. The humoral responses to the vaccine peptides were further characterized by the use of isotype-specific antibodies. The vaccine-specific serum antibodies in every subject were predominantly of the IgG class, indicative of an antigen-stimulated secondary immune response. Serum titres of the IgG antibodies rose over the period of vaccination in a similar fashion to the combined IgA, IgG and IgM responses. The peptide 2-specific serum antibodies were of the IgG1 subclass in subject 012 (Figure 1I), IgG1 and IgG3 in subjects 014 and 015 and IgG4 in subject 016 (data not shown). The peptide 5-specific serum antibodies were of the IgG3 subclass in subject 015 (endogenous response) and in subject 016 (Figure 1J). The peptide 8-specific serum antibodies were only detected at a relatively high serum dilution of 1:25 in subjects 012 and 015 (figure 1G and H) and were undetectable using the isotype-specific antibodies (data not shown).

One of the trial subjects (012) also had rising titres of serum anti-p53 antibodies (Figure 3A). As these antibodies were present at the baseline timepoint, it is not clear whether they were due to the vaccine or were the result of an increased antigen load following disease progression. Isotype-specific antibodies showed that the level of p53-specific IgG1 serum antibodies rose with increasing vaccinations, but the level of IgM antibodies remained at a constant level over the course of the vaccination schedule (Figure 3B).

A small proportion of the sera from the 30 normal and 54 cancer controls demonstrated weak reactivity to peptides in the vaccine although the absorbance in each case was less than that seen in the trial patients (Table 2). Serum reactivity to peptide 1 (3 subjects), peptide 3 (1 subject) and peptide 5 (2 subjects) were detected in the normal control group while responses to peptide 2 (1 subject), peptide 5 (3

subjects) and peptide 7 (1 subject) were seen in the non-trial cancer control population (Table 2). Six of the 54 cancer controls and one of the thirty normal controls had serum antibodies to p53 (Table 2). The p53 mutation status of the cancer controls was unknown.

Immunogen	Normal controls n = 30	Cancer controls n = 54	Trial subjects* n = 6
Peptide 1	3 (10%)	0	0
Peptide 2	0	1 (1.9%)	4 (66.7 %)
Peptide 3	1 (3.3%)	0	0
Peptide 4	0	0	0
Peptide 5	2 (6.7%)	3 (5.6%)	2^{e} (33.3 %)
Peptide 6	0	0	0
Peptide 7	0	1(1.9%)	0
Peptide 8	0	0	2 (33.3)
p53	1 (3.3 %)	6 (11.1%)	1^{en} (16.7%)

Table 2. Number of individuals with a humoral response to the vaccine peptides and p53. Sera from the cancer controls and trial subjects were tested at 1:25 dilution. Trial subjects were examined at a range of concentrations as shown in the text and Figure 1. Abbreviations: e endogenous responses to peptide 5 (subject 015), en endogenous response to p53 (subject 012). * In the trial subject group, a detectable response at any time point was considered positive.

Polyclonal sera with reactivity to peptides 2, 4, 5, 6 and 8 were obtained from both sheep while one animal did not respond to peptide 1 and neither animal responses to peptides 3 and 7 (Figure 2A). While serum antibody titres were equivalent for peptides 5 and 8, the response to peptide 2, 4, and 6 were on average 50% higher in the first immunized sheep. Given the method of immunization, it was not surprising that the serum anti-peptide titres in sheep were significantly higher than that found in the trial patients. For instance the sheep sera response to peptide 5 was still detectable at serum dilutions of 1:3200 while the highest serum titre in a trial subject was 1:200 to peptide 2 (Figures 2A and 1A). Interestingly the sheep with the highest titres of anti-peptide antibodies also had detectable and rising levels of vaccine-induced p53-specific antibodies. The p53-specific antibodies were measurable

at serum dilutions of 1:50 and 1:100, but then fell to background levels at higher dilutions (Figure 2B). Polyclonal sera against peptides 3 and 7 were successfully isolated from the immunised rabbits, but neither of the rabbits generated anti-p53 antibodies.

Cell-mediated response to the vaccine

All six of the trial patients who underwent *in vivo* DTH testing were found to have responses to the individual vaccine peptides (Table 3). The patients each responded to two, three or four of the peptides and responses were observed in at least one individual for all of the peptides, except for peptides 3 and 6. Peptides 5 and 2 were the most immunogenic for T cells with all six of the patients having a peptide 5-specific DTH response and five responding to peptide 2. There were three responses to peptide 8, two to peptides 1 and 7, and one response to peptide 4.

	Patient number					
Peptide	**012**	**014**	**015**	**016**	**017**	**018**
Peptide 1	Neg	Neg	Neg	104	24	Neg
Peptide 2	189	20	86	Neg	71	38
Peptide 4	Neg	Neg	13	Neg	Neg	Neg
Peptide 5	165	16	24	269	373	94
Peptide 7	16	Neg	Neg	39	Neg	Neg
Peptide 8	113	Neg	38	Neg	44	Neg
HIV-L2	Neg	Neg	Neg	Neg	Neg	Neg
Vehicle	Neg	Neg	Neg	Neg	Neg	Neg

Table 3. Extent of dermal induration seen in DTH testing of the six evaluable patients who completed all four vaccinations. Induration area (mm^2) was measured at 48 hours following injection with the individual vaccine peptides (10 g), HIV-L2 peptide (10 g) and vehicle alone 31.25% dimethyl sulfoxide (v/v), 0.05M NaCl. Neg indicates that induration (if any) was less than 10 mm^2.

Two of the six assessable trial patients (016 and 017) were found to have T cells specific for the vaccine, as measured by the proliferation assays. Patient 016 showed responses in the thymidine proliferation assay to peptide 1 (visit 5; stimulation index = 10.4; data not shown) and peptide 5 (visits 3, 4 and 5; stimulation index = 8.88, 64.6

and 22.2, respectively; Figure 4). This patient also showed a, peptide 5-specific proliferative response first appeared at visit 3 (1 month after-vaccination 2), and increased to maximal levels after three vaccinations had been administered.

The proliferative response to the vaccine pool was confirmed in subject 016 by CFSE proliferation assay (visit 4; 8.9 % of circulating CD3 and CD4 positive cells; Figure 5B), as was the response to peptide 5 (visit 5; 5.5 % of circulating CD3 and CD4 positive cells; data not shown). The other peptides were not tested individually due to limiting cell numbers. Subject 017 also had a specific proliferative response to the vaccine peptide pool measured by CFSE proliferation assay (visit 5; 3.9 % of circulating CD3 and CD4 positive cells; Figure 5D). Both of these vaccine-specific proliferation assays can be attributed to the vaccination schedule as they were not present at baseline timepoints (Figure 4, Figure 5A and 5C). Also, the PBMCs from patients 016 and 017 did not proliferate in the absence of stimulation with either the vaccine pool or peptide 5 (data not shown). Due to limiting cell numbers, only two other patients were tested by CFSE proliferation assay (012 and 015) and neither of them had a measurable vaccine-specific proliferative response.

In contrast to the proliferation assays, none of the trial patients were found to have vaccine-specific IFN-γ secreting T cells measurable by ELISpot assay (data not shown). Five of the patients were tested for responses to CMV lysate and four of the five demonstrated a reproducible specific response to CMV lysate confirming that the cells had been incubated at 37 °C for a sufficient time for a whole protein antigen to be processed and presented in the context of the MHC class I or II pathways. There was no measurable cell-mediated response to p53 in any of the trial subjects as assessed by thymidine proliferation assay, CFSE proliferation assay (only subjects 012, 015, 016 and 017 tested) or IFN-γ ELISpot assay.

None of the cancer controls had a measurable cell-mediated response to the vaccine peptides assayed by thymidine proliferation assay (9 subjects), CFSE proliferation assay (4 subjects) or IFN-γ ELISpot (9 subjects). Similarly, none of the normal controls had a measurable response to p53 assayed by thymidine proliferation assay (3 subjects), or CFSE proliferation assay (2 subjects).

Discussion

In this study we have demonstrated that the CDR regions of human anti-p53 antibodies are capable of initiating humoral and cellular immunity in animals and in individuals with advanced malignancy. Furthermore, anti-p53 antibodies were

observed in a sheep and in one individual, suggesting that an idiotype immune cascade may have been triggered by the vaccinations.

A clinically effective immune response to the self-protein p53 requires the production of specific cytotoxic T cells. We have sought to elicit such a response by immunizing with Ab1 (CDR regions from anti-p53 antibodies), and thereby inducing the production of anti-idiotype antibodies (anti-peptide antibodies or Ab2) that are an immunological mimic of p53. In animal models, these anti-idiotype antibodies (Ab2) have been shown to represent a surrogate p53 antigen which can lead to the *in vivo* production of anti-p53 cytotoxic T cells[24, 25]. Our strategy differs from previous human idiotype trials in that we have vaccinated subjects with Ab1 rather than anti-idiotype antibodies (Ab2) isolated from immunized animals[22, 26-29].

To be effective, the peptide vaccine must first be presented to CD4 positive T cells in the context of the MHC class II pathway. With T cell help, peptide specific B cells must then undergo isotype switching to produce a panel of anti-idiotype (Ab2) antibodies[30]. Clearly this process has occurred in four of the six evaluable subjects in this study, and by inference the T helper cell response is likely to be of the Th2 subtype. Evidence supporting this contention is the finding of predominantly IgG1 and IgG3 anti-peptide antibodies, and the identification of vaccine-specific T cells which failed to secrete IFN-γ. Interestingly, only three of the eight peptides (2, 5 and 8) were responsible for the observed humoral and cell mediated immune response. There are a number of possible explanations for the lack of immunogenicity of the remaining peptides, including the format and timing of vaccinations, and their HLA compatibility with the subjects in this study[31-33]. One of the most likely reasons is that since the peptides are derived from entirely human immunoglobulin sequences, the particular T and B cells responsible for recognition of these self sequences may have been deleted during clonal selection[34]. Certainly the studies of Ruiz and colleagues have demonstrated that only a subset of the mouse anti-p53 CDRs are immunogenic in mouse models[24, 25].

Given that vaccinated subjects were able to produce antigen-specific Ab2, we hypothesized that a proportion of these Ab2 molecules could trigger an anti-p53 (Ab3) response. Indeed this was the case in one of two vaccinated sheep, and in one of the patients, as demonstrated by rising titres of p53-specific antibodies. With regard to the sheep, the p53-specific response was clearly induced by the vaccine, since it was not detectable in the pre-immune serum, and correlated well with the high titres of vaccine-specific antibodies. On the other hand, the significance of the anti-p53 response in the trial subject remains uncertain. This individual had an endogenous

p53-specific immune response at baseline, and while the titre of anti-p53 antibodies increased over the course of the vaccinations, the tumor burden was also increasing over this period.

On the basis of these findings, it seems reasonable to conclude that the current vaccination strategy was not optimal in inducing detectable anti-p53 immune responses, at least in humans. There are a number of explanations for this finding which warrant further consideration. Firstly, it is important to consider the possibility that the *in vitro* assays used in this study failed to detect humoral and CD8+ T cell responses to p53. While this seems unlikely, it is true that reactivity to conformational epitopes on recombinant p53 would not be detected in the ELISA. Furthermore, in the absence of cross priming, the T cell assays would only identify CD4+ responses, since the p53 was added exogenously and presented in association with MHC class II by antigen presenting cells. Detection of a CD8 positive response to p53 would require the *in vitro* use of short overlapping peptides (9 to 13 amino acids in length) which span the length of p53[35].

These technical issues aside, it is important to identify those characteristics of Ab2 which will produce anti-p53 antibodies (Ab3). In this regard, we suggest that the absolute amount of Ab2 available for antigen presentation may impact on its potential role as an immunogen. The highest titers of Ab2 were observed in the sheep with an anti-p53 antibody response. The second sheep, which had much lower titers of vaccine-specific antibodies, correspondingly showed no detectable p53- specific humoral immunity. While the titers of Ab2 in the trial patients were many fold lower than those seen in either sheep, it is interesting to note that the patient with the highest Ab2 response was also the individual in whom there was demonstrable anti-p53 reactivity.

Another factor which may have influenced the development of Ab3 was the time taken to develop anti-peptide or Ab2 responses a factor that will in turn be dependent upon the duration of vaccination. Evaluable patients did not develop peptide immunity until after the third or fourth vaccination, and the titer of antibody fell once vaccinations were discontinued. It is therefore possible that anti-p53 antibodies may only be generated following repeated vaccinations over a sustained period of time. Comparison with other vaccine trials suggests that peptides are best administered at weekly intervals for 4 weeks, followed by regular boosts over a period of many months[36-38]. Indeed since p53 is self protein, immune responses are unlikely to have good memory and therefore vaccinations may need to be continued indefinitely.

A final possibility which may explain the failure to induce anti-p53 antibodies is that the epitopes on Ab2 may mimic regions of p53 which are not recognised by the immune system. Since the idiotype network is designed to generate topochemical copies of antigenic epitopes[39], and the vaccine was derived from individuals with specific human anti-p53 antibodies, this explanation seems less likely.

Irrespective of the mechanism involved, in order to avoid difficulties that may be encountered in production of sufficient the levels of vaccine-specific antibodies an alternative is to isolate and immunize with vaccine-specific antibodies (Ab2), thus eliminating one step in the idiotype cascade. A further advantage of this approach is that it will allow the design of vaccines which immediately elicit the production of anti-p53 antibodies, and more importantly CD8+ T cell cells[40, 41]. Considering the potential time taken to complete all steps of the idiotype cascade, further immunization with Ab1 may not be prudent, especially in the adjuvant setting where the induction of anti-tumor immunity immediately following surgery is important.

In conclusion, this study demonstrates that vaccinating with human antibody CDR regions represents a novel method for inducing human Ab2, and in turn suggests that isolation of these antibodies could yield a useful immunogen, particularly in the adjuvant setting.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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CLAIMS:

1. An idiotypic vaccine composition, the vaccine composition comprising a pharmaceutically acceptable carrier and at least one peptide, wherein the at least one peptide is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASKKG-Y_2 and X_3-MQGLQTPYT-Y_3 in which X_1, X_2, X_3, Y_1, Y_2 and Y_3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

2. The composition according to claim 1 wherein X_1 is absent or is AVYYC, X_2 is absent or is LEWVG, X_3 is absent or is GVYYC, Y_1 is absent or is WGQGT, Y_2 is absent or is RVTI and Y_3 is absent or is FGEGT.

3. The composition according to claim 1 or 2 wherein the at least one peptide is selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

4. The composition according to any preceding claim wherein the composition comprises at least 2 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

5. The composition according to any preceding claim wherein the composition comprises all 3 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

6. The composition according to any preceding claim wherein the composition further comprises at least one peptide selected from the group consisting of LEWMGIINPSGGSANYAPKFKGRLTMS, KLLIHWASTRESGVPDR, AGLFCQQYYTTPLTFGGGT, YFCSRVKAGGPDYWGQGT and LLIYLGSTRASGVPDR.

7. The composition according to any preceding claim wherein the peptide is an analogue of a peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

8. The composition according to any preceding claim wherein the composition further comprises an adjuvant.

9. The composition according to any preceding claim wherein the adjuvant is selected from the group consisting of cytokines, immune stimulatory complexes (ISCOMS), CpG oligonucleotides, lipopolysaccharide, muramyl dipeptides, bacterial toxins such as diptheria, pertussis and tetanus toxins, ovalbumin, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmit-oyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine, RIBI, aluminium potassium sulfate (alum), beryllium sulfate, silica, kaolin,

carbon, water-in-oil emulsions, oil-in-water emulsions, *Corynebacterium parvum*, *Bordetella pertussis*, polyribonucleotides, sodium alginate, lanolin, lysolecithin, vitamin A, saponin, liposomes, levamisole, DEAE-dextran, blocked copolymers or other synthetic adjuvants including Merck Adjuvant 65, or Freund's Incomplete Adjuvant and Complete Adjuvant.

10. The composition according to claim 9 wherein the adjuvant is granulocyte-macrophage colony stimulating factor (GM-CSF).

11. An idiotypic vaccine composition, the vaccine comprising a pharmaceutically acceptable carrier and at least one peptide, the at least one peptide being characterised in that it competes with a peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT for binding to p53.

12. An idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one peptide, the at least one peptide being characterised in that an antibody raised against the peptide reacts with at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

13. The composition according to claim 11 or 12 wherein the peptide is derived from the complementarity determining region of a human anti-p53 antibody.

14. The composition according to any one of claims 11 to 13, wherein the at least one peptide is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASKKG-Y_2 and X_3-MQGLQTPYT-Y_3 in which X_1, X_2, X_3, Y_1, Y_2 and Y_3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

15. The composition according to claim 14 wherein X_1 is absent or is AVYYC, X_2 is absent or is LEWVG, X_3 is absent or is GVYYC, Y_1 is absent or is WGQGT, Y_2 is absent or is RVTI and Y_3 is absent or is FGEGT.

16. The composition according to any one of claims 11 to 15 wherein the at least one peptide is selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

17. The composition according to any one of claims 11 to16 wherein the composition comprises at least 2 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

18. The composition according to any one of claims 11 to 17 wherein the composition comprises all 3 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

19. The composition according to any one of claims 11 to 18 wherein the composition further comprises at least one peptide selected from the group consisting of LEWMGIINPSGGSANYAPKFKGRLTMS, KLLIHWASTRESGVPDR, AGLFCQQYYTTPLTFGGGT, YFCSRVKAGGPDYWGQGT and LLIYLGSTRASGVPDR.

20. The composition according to any one of claims 11 to 19 wherein the peptide is an analogue of a peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

21. The composition according to any one of claims 11 to 20 wherein the composition further comprises an adjuvant.

22. The composition according to claim 21 wherein the adjuvant is selected from the group consisting of cytokines, immune stimulatory complexes (ISCOMS), CpG oligonucleotides, lipopolysaccharide, muramyl dipeptides, bacterial toxins such as diptheria, pertussis and tetanus toxins, ovalbumin, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmit-oyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine, RIBI, aluminium potassium sulfate (alum), beryllium sulfate, silica, kaolin, carbon, water-in-oil emulsions, oil-in-water emulsions, *Corynebacterium parvum*, *Bordetella pertussis*, polyribonucleotides, sodium alginate, lanolin, lysolecithin, vitamin A, saponin, liposomes, levamisole, DEAE-dextran, blocked copolymers or other synthetic adjuvants including Merck Adjuvant 65, or Freund's Incomplete Adjuvant and Complete Adjuvant.

23. The composition according to claim 22 wherein the adjuvant is granulocyte-macrophage colony stimulating factor (GM-CSF).

24. An idiotypic vaccine composition, the vaccine comprising a pharmaceutically acceptable carrier and at least one DNA molecule, the DNA molecule comprising a sequence encoding at least one peptide, wherein the at least one peptide is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASKKG-Y_2 and X_3-MQGLQTPYT-Y_3 in which X_1, X_2, X_3, Y_1, Y_2 and Y_3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

25. The composition according to claim 24 wherein X_1 is absent or is AVYYC, X_2 is absent or is LEWVG, X_3 is absent or is GVYYC, Y_1 is absent or is WGQGT, Y_2 is absent or is RVTI and Y_3 is absent or is FGEGT.

26. The composition according to claim 24 or 25 wherein the composition comprises a DNA molecule encoding at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

27. The composition according to any one of claims 24 to 26 wherein the composition comprises least 2 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

28. The composition according to any one of claims 24 to 27 wherein the composition comprises all 3 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

29. The composition according to any one of claims 24 to 28 wherein the DNA molecule comprises a further sequence encoding GM-CSF.

30. The composition according to any one of claims 24 to 29 wherein the DNA molecule comprises a further sequence encoding LEWMGIINPSGGSANYAPKFKGRLTMS, KLLIHWASTRESGVPDR, AGLFCQQYYTTPLTFGGGT, YFCSRVKAGGPDYWGQGT and LLIYLGSTRASGVPDR.

31. The composition according to any one of claims 24 to 30 for use in DNA vaccination.

32. An idiotypic vaccine composition, the vaccine comprising a pharmaceutically acceptable carrier and at least one DNA molecule, the DNA molecule comprising a sequence encoding at least one peptide, the at least one peptide being characterised in that it competes with a peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT for binding to p53.

33. An idiotypic vaccine composition, the composition comprising a pharmaceutically acceptable carrier and at least one DNA molecule, the DNA molecule comprising a sequence encoding at least one peptide, the at least one peptide being characterised in that an antibody raised against the peptide reacts with at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

34. The composition according to claim 32 or 33 wherein the peptide encoded by the DNA molecule is derived from the complementarity determining region of a human anti-p53 antibody.

35. The composition according to any one of claims 32 to 34 wherein the peptide encoded by the DNA molecule is selected from the group consisting of X_1-LLQALKH-Y_1, X_2-FIRSKAYGAATAYAASKKG-Y_2 and X_3-MQGLQTPYT-Y_3 in which X_1, X_2, X_3, Y_1, Y_2 and Y_3 are independently either absent or an amino acid sequence of preferably less than 10 amino acids which provides a framework for the specified peptide.

36. The composition according to claim 35 wherein X_1 is absent or is AVYYC, X_2 is absent or is LEWVG, X_3 is absent or is GVYYC, Y_1 is absent or is WGQGT, Y_2 is absent or is RVTI and Y_3 is absent or is FGEGT.

37. The composition according to any one of claims 32 to 36 wherein the composition comprises a DNA molecule encoding at least one peptide selected from the group consisting of AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

38. The composition according to any one of claims 32 to 37 wherein the composition comprises least 2 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

39. The composition according to any one of claims 32 to 38 wherein the composition comprises all 3 of the peptides AVYYCLLQALKHWGQGT, LEWVGFIRSKAYGAATAYAASMKGRVTI, and GVYYCMQGLQTPYTFGEGT.

40. The composition according to any one of claims 32 to 39 wherein the DNA molecule comprises a further sequence encoding GM-CSF.

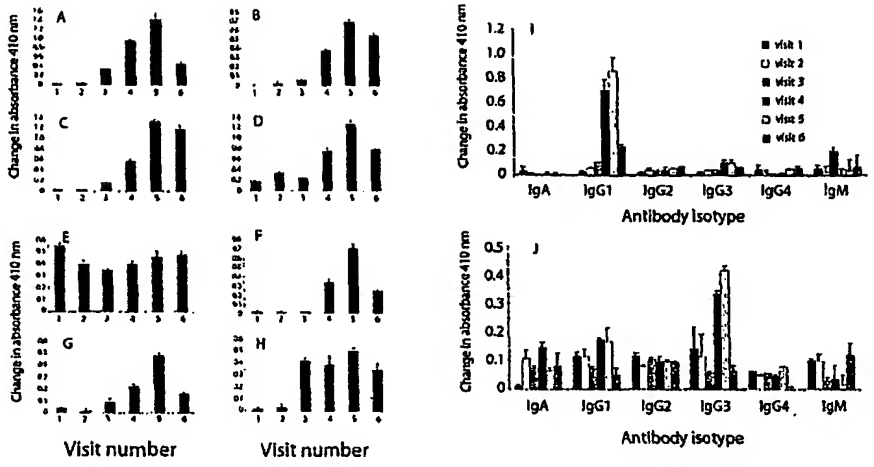
41. The composition according to any one of claims 32 to 40 wherein the DNA molecule comprises a further sequence encoding LEWMGIINPSGGSANYAPKFKGRLTMS, KLLIHWASTRESGVPDR, AGLFCQQYYTTPLTFGGGT, YFCSRVKAGGPDYWGQGT and LLIYLGSTRASGVPDR.

42. The composition according to any one of claims 32 to 41 for use in DNA vaccination.

43. A method of inducing an anti-p53 idiotypic response in a subject, the method comprising administering to the subject the composition according to any one of claims 1 to 42.

44. A method of inducing immunity against a disease caused by expression of mutant p53, the method comprising administering to the subject the composition according to any one of claims 1 to 42.

Figure 1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B
C
D
E
F
G
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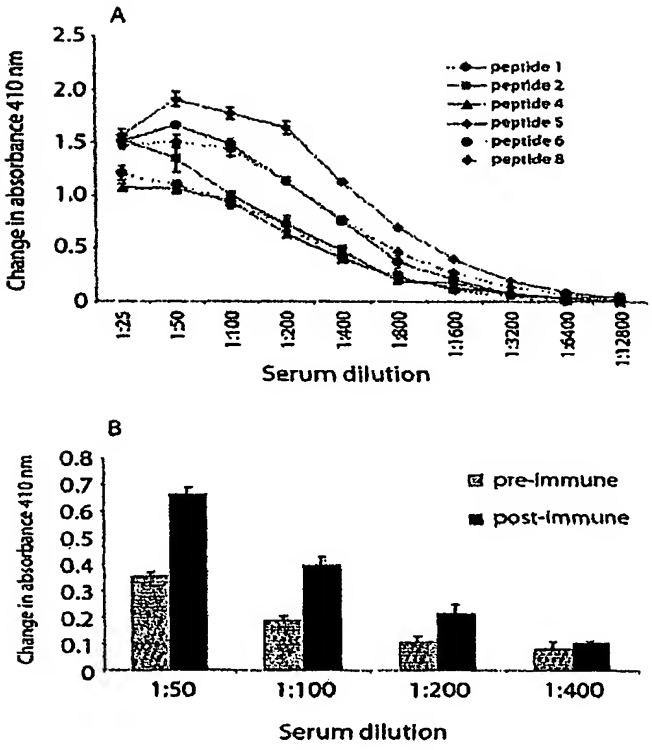
Change in absorbance 410 nm
Visit number
Visit number
I
J
visit 1
visit 2
visit 3
visit 4
visit 5
visit 6
IgA
IgG1
IgG2
IgG3
IgG4
IgM
Antibody isotype
Antibody isotype

Figure 2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Change in absorbance 410 nm
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2.0
1.5
1.0
0.5
0
peptide 1
peptide 2
peptide 4
peptide 5
peptide 6
peptide 8
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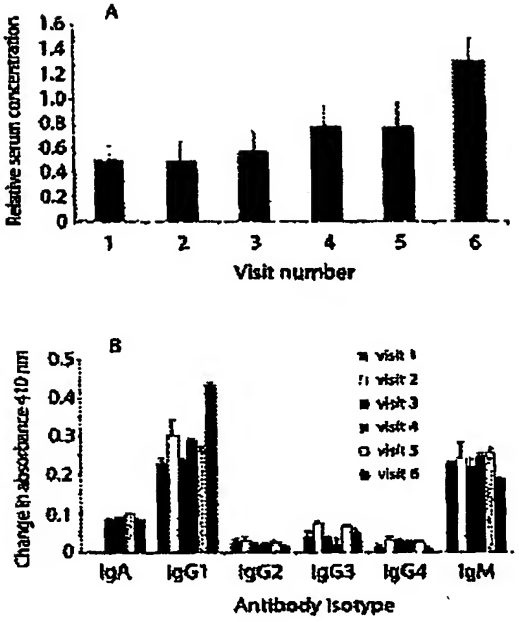
1:50
1:100
1:200
1:400
1:800
1:1600
1:3200
1:6400
1:12800
Serum dilution
B
Change in absorbance 410 nm
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
pre-immune
post-immune
1:50
1:100
1:200
1:400
Serum dilution

Figure 3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Relative serum concentration
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0.2
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1
2
3
4
5
6
Visit number
B
Change in absorbance 410 nm
0.5
0.4
0.3
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0.1
0
IgA
IgG1
IgG2
IgG3
IgG4
IgM
Antibody Isotype

Figure 4

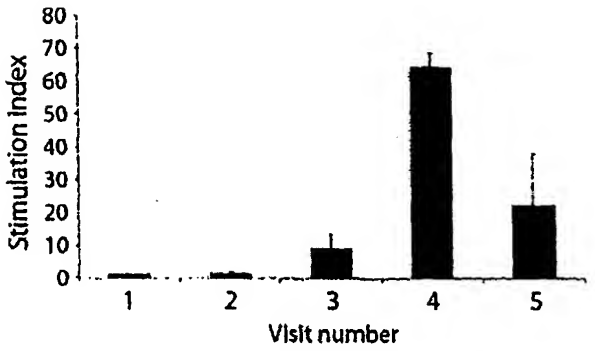
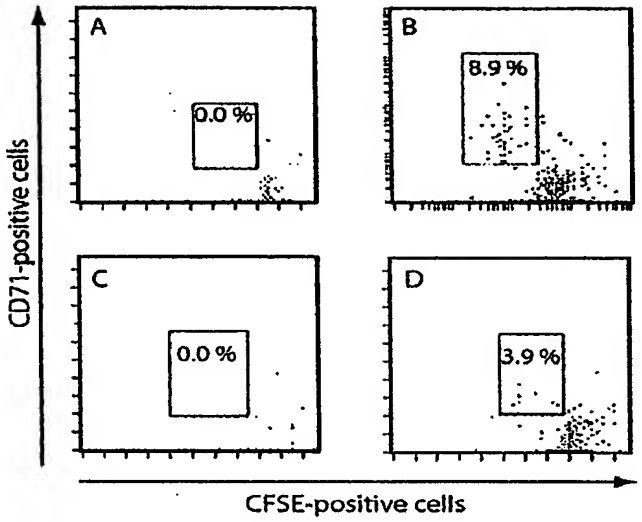


Figure 5



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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/000225

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K 16/18, 16/44; A61K 39/395; A61P 37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See electronic databases consulted below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See electronic databases consulted below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Registry, CA. Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2000/056770, (ST. VINCENT'S HOSPITAL SYDNEY LIMITED) 28 September 2000 See whole document, in particular SEQ ID NO 34, 36, 38, 40, 42, 44, 50, 52, 58 and 60.	1-44
X	(D.W.J. COOMBER ET AL) "Generation of Anti-p53 Fab Fragments from Individuals with Colorectal Cancer". Journal of Immunology, 1999, vol. 163, pages 2276-2283. See whole document, in particular Figure 4, amino acid sequence of heavy chain clones 163.9, 163.20, 163.1, 163.5, 163.14, 163.2, 163.6, 163.17, 163.24	1-44
X	(D.W.J. COOMBER ET AL) "Isolation of Human Antibodies against the Central DNA Binding Domain of p53 from an Individual with Colorectal Cancer Using Antibody Phage Display". Clinical Cancer Research, 2001, vol. 7, pages 2802-2808. See whole document, in particular Figure 6, amino acid sequence of Fab 1159.8; discussion.	1-44

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 April 2004	- 1 JUN 2004
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer SWARUP CHATTERJEE Telephone No : (02) 6283 2259

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/000225

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

      [X] a sequence listing

      [ ] table(s) related to the sequence listing

   b. format of material

      [X] in written format

      [ ] in computer readable form

   c. time of filing/furnishing

      [X] contained in the international application as filed

      [ ] filed together with the international application in computer readable form

      [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2004/000225

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2000/056770	AU	31353/00	CA	2365104	EP	1171469

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

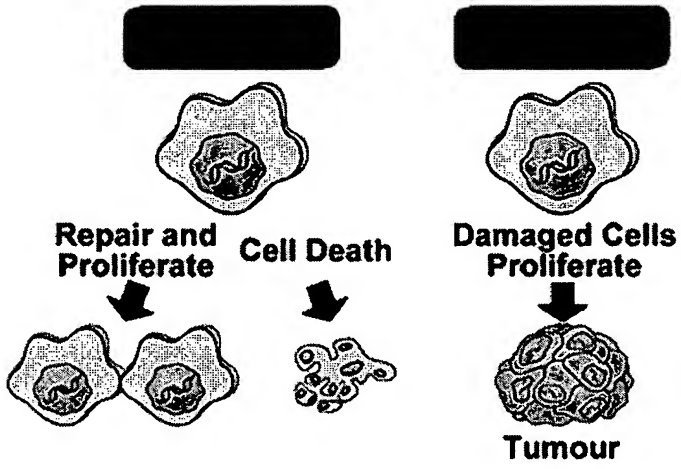
END OF ANNEX

The Pentrys™ vaccine is a unique treatment applicable to up to 50% of all cancers. It targets one of the most common defects in cancer cells - a mutated p53 gene. Pentrys™ could be an effective treatment for patients following removal of a tumour and could also be used in cases where early diagnosis is possible.

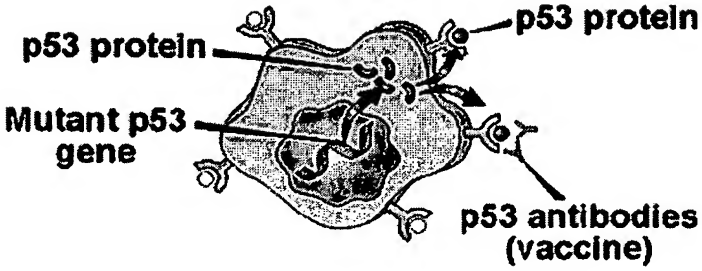
Background - What is p53 and why is it important in cancer?

p53 is an important gene for regulating the division of damaged cells. It acts as a brake in the cell cycle, stopping cells which have damaged DNA from multiplying and inducing cell death. In up to 50% of cancer cases p53 is damaged or mutated. This inhibits the function of p53, which means that the stop signal for cell division is removed. This allows damaged cells to divide uncontrollably and results in the formation of a cancerous tumour.

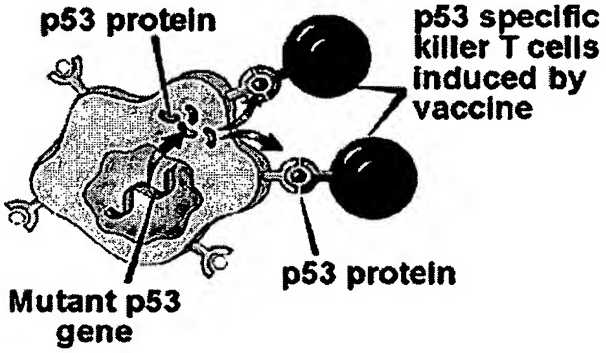
When the p53 gene is mutated it produces abnormally large quantities of the p53 protein. Fragments of the mutated p53 are displayed on the outer surface of the cell in conjunction with histocompatibility antigens. This does not occur with undamaged p53 and therefore provides a point of difference between normal and cancer cells. Such a difference could induce an immune response against the cancerous cells if the immune system could be tricked into recognizing them as foreign rather than self. The immune system only responds to cells or molecules which it recognizes as being foreign to the body.



1. Carcinogen/Radiation damages DNA



2. Only tumour cells display p53 proteins



3. Vaccine tricks T-Cells to attack

The Pentrys™ Vaccine Technology

To be able to take advantage of the distinction that is made between cancer cells and normal cells by the mutated p53 protein, AustCancer has developed a technology known as an anti-idiotypic vaccine against mutated p53. An anti-idiotypic vaccine is designed to trick the body into seeing the cancerous cells as being foreign. This induces an immune response and those cells displaying mutant p53 protein on their surface are killed by the immune system. Since the mutated p53 protein is only displayed on tumour cells, only tumour cells will be killed by the immune system. Normal cells will be unaffected.

Excellent results from anti-idiotypic p53 technology have previously been generated in mouse systems demonstrating that this strategy is feasible for developing an effective cancer vaccine for humans.

The p53 Human Antibodies

Pentrys™ vaccine technology is based on the synthesis of human antibodies to the mutated p53 tumour suppressor gene. The antibodies that these are derived from were isolated from the lymph nodes of individuals who had shown a strong natural immune response to their cancer and recovered unexpectedly.

The antibodies were developed after seven years of research by a St Vincent's Hospital, Sydney team led by Associate Professor Robyn

Ward. The antibodies are unique, as they are the only entirely human p53 antibodies currently available in the world.

The Pentrys™ vaccine is a mixture of nine peptides (small proteins), which are derived from these antibodies.

What makes Pentrys™ different??

Pentrys™ has some distinct competitive advantages over other cancer vaccine approaches. Firstly, it is a broad-spectrum vaccine which is potentially applicable to the 50% of all cancers which have mutations in their p53. gene. This includes common cancers such as breast, bowel, prostate and lung. Most cancer treatments are only for specific cancer types and are unlikely to be effective against such a broad range of cancers.

The second major point of difference is that many cancer vaccines currently in development involve removing a patient's own cells and engineering them to trick that particular person's immune system into attacking the cancerous cells.

Pentrys™ is not patient specific and does not involve the use of cells. The same vaccine can be used in all patients. This decreases the time taken to prepare the treatment and will also make it more cost-effective and less complex to obtain regulatory approvals than competing technologies.

Being a broad-spectrum vaccine applicable to up to 50% of all cancers in all patients Pentrys™ has the potential to become a blockbuster therapy.

Clinical Trial

A Phase 1b/2a clinical trial on the Pentrys™ vaccine in humans was successfully completed at St Vincent's Hospital, Sydney. A Phase 1a trial on four patients was completed earlier this year with no evidence of drug related toxicity and initial evidence that the drug is immunogenic.

The St Vincent's Hospital Clinical Trials Centre conducted the Phase 1b/2a study which confirmed the safety of the vaccine and demonstrated a strong immune response in all 14 patients involved in the trial. The range of cancers represented in the trial reinforced the potential applicability of Pentrys™ to up to 50% of all cancers. Additional patents are to be lodged as a result of knowledge gained from the trial.

The Company has commenced planning for a full Phase 2b study to follow in the second half of 2003.

ASX Announcement, **AustCancer's Galenica Signs Materials Transfer Agreement with Memorial Sloan-Kettering in New York** 22-Jun-2004

ASX Announcement, **AustCancer to Acquire U.S. Vaccine Developer** 20-May-2004

ASX Announcement, **Phase II Cancer Vaccine Trial Underway** 30-Apr-2004

ASX Announcement, **Phase II Pentrix Trial to Commence** 13-Apr-2004

ASX Announcement, **CFO appointment** 16-Mar-2004

ASX Announcement, **AustCancer to list on NASDAQ** 20-Feb-2004

ASX Announcement, **USA Scientists Strengthen AustCancer Advisory Board** 22-Jan-2004

ASX Announcement, **Phase II Anti-Cancer Vaccine Trial Approved** 19-Dec-2003

ASX Announcement, **PentrixTM Presented at Major US Cancer Conference** 22-Jul-2003

ASX Announcement, **Phase II Trials for Anti-Cancer Vaccine** 2-Jul-2003

ASX Announcement, **AustCancer and BresaGen Collaborate on Cancer Vaccine Trial** 8-May-2003

ASX Announcement, **Successful Completion of Pentrix Clinical Trials at St Vincent's Hospital** 26-Feb-2003

ASX Announcement, **Share Placement** 16-Jan-2003

ASX Announcement, **Commercial Strategy Following Successful Clinical Trials** 15-Jan-2003

ASX Announcement, **Commercialisation of the Pentrix Anti-Cancer Vaccine** 12-Dec-2002

Presentation, **Technical Presentation: Pentrix - Phase 1b/2a Clinical Trial** 4-Dec-2002

ASX Announcement, **Strong Results from St Vincent's Hospital Cancer Vaccine Human Trials** 12-Nov-2002

ASX Announcement, **Pentrix Cancer Vaccine Clinical Trial Advances to Evaluation Stage** 18-Sep-2002

ASX Announcement, **Pentrix Anti-Cancer Vaccine Clinical Trials Progressing Rapidly** 30-Apr-2002

ASX Announcement, **Major Cancer Trial Commenced** 21-Feb-2002

ASX Announcement, **Pentrix Anti-Cancer Vaccine - Clinical Trial Successfully Completed** 30-Jan-2002

ASX Announcement, **Publication of Pentrix Technology in International Journal** 25-Oct-2001

ASX Announcement, **p53 Patent Moves to International Phase** 9-Oct-2001

ASX Announcement, **Clinical Trials for Pentrix (p53) Cancer Vaccine Commenced** 2-Oct-2001

ASX Announcement, **Top Scientist Appointed Executive Director** 18-Sep-2001

Presentation, **Technical Presentation - Pentrix: Assays for Effectiveness of Immunotherapy** 2-Aug-2001

ASX Announcement, **Progress on p53 cancer vaccine project announced** 11-Apr-2001

Presentation, **Technical Presentation - Pentrix Cancer**

Vaccine 31-Mar-2001

ASX Announcement, **Raising of Working Capital for Cancer Vaccine Trials** 19-Mar-2001

ASX Announcement, **Australian Cancer Technology focus on leading edge drug development** 6-Mar-2001

ASX Announcement, **Exodus acquires cancer vaccine project** 8-Feb-2001

Exhibit C

Annals of Oncology

Annals of Oncology 15:324-329, 2004

Original Paper

Phase I clinical trial of a human idiotypic p53 vaccine in patients with advanced malignancy

Received 1 May 2003; revised 18 August 2003; accepted 29 September 2003;

Background:

The purpose of this study was to induce immunity to p53 by using an idiotypic vaccine, composed of a pool of eight peptides derived from the complimentarity determining regions (CDRs) of human anti-p53 antibodies.

Patients and methods:

Subjects with advanced malignancy received up to four, monthly intradermal injections of pooled peptides (500 μg of each) admixed with granulocyte–macrophage colony-stimulating factor (GM-CSF; 100 μg). In addition, two sheep and two rabbits were also vaccinated with the pooled peptides.

Results:

Fourteen subjects were enrolled into the study and six of these completed the vaccination schedule. The vaccine was well tolerated by all subjects and no major adverse events were attributable to the vaccine. All subjects mounted *in vivo* delayed type hypersensitivity (DTH) responses to two or more of the individual vaccine peptides. Vaccine-induced antibodies specific for peptides 2, 5 or 8 were detected in four of six subjects, and two of these had vaccine-specific, cell-mediated responses. Increasing titers of p53-specific antibodies were found in one patient. No T-cell response to p53 was observed in any of the subjects. All animals developed humoral immunity to the peptides and one of the sheep developed rising serum titers of anti-p53 antibodies.

Conclusions:

Vaccination with human antibody CDR regions represents a novel method for inducing human antibodies, which may in turn serve as immunological mimics of p53.

M. Lomas[1], W. Liauw[1,2], D. Packham[1], K. Williams[2], A. Kelleher[3], J. Zaunders[3] and R. Ward[1,4,*]

[1] Department of Medical Oncology, [2] Clinical Trials Unit and [3] Centre for Immunology, St Vincent's Hospital, Darlinghurst; [4] School of Medicine, University of New South Wales, Sydney, Australia

Key words: cancer, idiotype, p53, vaccination